academicJournals

Vol. 9(3), pp. 43-47, 22 January, 2015
DOI: 10.5897/AJPP2014. 4227
Article Number: C36078550326
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Determination of the abortifacient activity of the aqueous extract of *Phytolacca dodecandra* (L'Her) leaf in Wistar rats

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Received 11 November, 2014; Accepted 21 January, 2015

Throughout history women have tried to control their fertility using herbal remedies; traditionally *Phytolacca dodecandra* leaves have been used as an abortifacient. The objective of this study was to determine the abortifacient activity of the aqueous extract of *P. dodecandra* leaf in Wistar rats. Hundred grams of shaded dried powdered leaves were soaked in 0.5 L of cold water, and using cold maceration, an aqueous extract was obtained. Acute toxicity was carried out using Lorke's method. The abortifacient activity of the plant extract was tested using a modified method. Thirty pregnant rats were randomly distributed into five groups each consisting of 6 rats and were treated. The percentage number of rats that aborted per treated group was compared with those of the controls. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, phenolics, steroids and triterpenoids. Toxicity signs such as reduced appetite, sleepiness, shivering and excessive urination were observed at a dose of 2048 mg/kg; however, no deaths were observed. In the groups in which 125 and 250 mg/kg of *P. dodecandra* extract was administered, 83.3% of the rats aborted, and in the group in which 500 mg/kg was administered, 100% of the rats aborted. This study has substantiated the abortifacient activity of the aqueous extract of *P. dodecandra* leaves which may be attributed to the phytochemicals.

Key words: Abortifacient, phytochemical screening, acute toxicity, *Phytolacca dodecandra*.

INTRODUCTION

Traditional medicine usage in rural Ugandan population for day-to-day healthcare need is close to 90% (Kamatenesi et al., 2006). Despite the limited availability of safety and efficacy information about a number of herbal medicinal preparations, dispensing and use of these products is one of the common health care practices in Uganda (Lutoti et al., 2013). Throughout history, women have tried to control or enhance their fertility. Many herbal remedies are traditionally used as contraceptives (to prevent the ovulation or fertile-zation), abortifacients (to prevent implantation) and emmena-gogues (to prevent uterine flow) or oxytocics (to stimulate

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uterine contractions, particularly to promote labour) (Ritchie, 2001). Abortion is the termination of pregnancy by removal or premature expulsion from the uterus of a fetus or embryo prior to viability (Grimes et al., 2006). Abortion may be due to maternal exposure to chemicals, which can disrupt pregnancy and cause detachment of the embryo (Feranada et al., 2000). Currently, the use of herbs to terminate pregnancy by many young women of reproductive age is high yet some of these herbs often need to be used in doses that may be toxic to the woman or even potentially fatal (Selin, 1997; Kress, 2013). Others do not work but rely on the fact that spontaneous abortion is common; in such cases, the occurrence of the abortion is attributed to the herbs if any were being used (Selin, 1997; Kress, 2013).

Traditionally, in Central and Western Uganda, the aqueous extract of *Phytolacca dodecandra* is used to terminate pregnancy within the first six to eight weeks after the last menstrual period (Prada et al., 2005; Selin, 1997). Little information is available on the pharmacological properties and toxicity of abortifacient herbal preparations in Uganda, yet the use of herbs to terminate pregnancy is high and sometimes with serious consequences including death.

Therefore, there is an urgent need to determine and document the abortifacient activity of such herbs and also determine the acute toxicity.

In addition, screening of plants with abortifacient activity and subsequent identification and characterization of the active principle will prove to be useful guide towards the formulation of cheaper, affordable contraceptives with reduced toxicity (Dabhadkar and Zade, 2013).

This therefore provides an avenue for the current study to evaluate the abortifacient activity of the aqueous extract of the leaves of *P. dodecandra*.

P. dodecandra is a climbing or scrambling dioecious, semi-succulent shrub sometimes a liana with glabrous stems up to 10 to 20 m long (Zimudzi, 2007). In traditional medicine, the plant is to treat various ailments such as diarrhea, intestinal infestation, skin infections and dysmenorrhea in humans and animals (Nalule et al., 2011).

The leaf extract of *P. dodecandra* contains terpenoids, phenolics and alkaloids (Ogutu et al., 2012). According to katende et al. (1995), *P. dodecandra* is a very poisonous plant in both people and animals. The leaves, roots and seeds of *Phytolacca acinosa*, a plant from the same family as *P. dodecandra* were found to have abortifacient activity by causing uterine stimulation in rats (Yeung et al., 1987). Therefore, the aim of this study was to determine the abortifacient activity of *P. dodecandra* leaves.

Screening of plants with abortifacient activity and subsequent identification and characterization of the active principle is a useful guide towards the formulationof cheaper, affordable contraceptives with reduced toxicity (Dabhadkar and Zade, 2013).

MATERIALS AND METHODS

The materials and equipment used for carrying out the experiment included 40 female Wistar rats aged three months, *P. dodecandra* leaves, misoprostol tablets (200 mcg), HCG strips, intragastric rubber cannulas, distilled water, steam bath, phytochemical screening reagents of analytical grade, glassware, oven, surgical blades, Analytical balance, blender, Vacuum pump filter, cages and fridge.

Study site

The study was carried out in the pharmaceutical chemistry laboratory, biochemistry laboratory and Animal Research Facility of Mbarara University of Science and Technology.

Study design

This was an experimental short term prospective research study.

Plant collection and extract preparation

Healthy mature plant leaves were collected in January from Wakiso district, Uganda (0.4000°N, 32.4833°E), specimen taken for identification at the Department of Botany, Faculty of Science, Mbarara University of Science and Technology, and was given a Voucher Number: Angella Namulindwa 001. The plant leaves were washed and shade dried for 14 days at room temperature, then pulverized using a blender. 100 g of the leaf powder were soaked in 0.5 L of cold distilled water for 48 h at room temperature with intermittent shaking to allow complete extraction. The mixture was filtered using a vacuum pump filter with Whatman filter paper No. 1 to obtain the filtrate. The filtrate was then concentrated to a constant weight using an oven maintained at temperature of 50°C.

Experimental animals

Female Wistar rats (155 to 280 g) were purchased from the animal facility of Mbarara University of Science and Technology. The animals were initially acclimatized to the laboratory conditions for one week prior to the experiment. The animals were housed in appropriate cages under a 12 h light:dark cycle and were allowed free access to standard rat feeds and clear drinking water *ad libitum*. Animals were handled in this study as per the National Institute of Health guidelines (2011) for the care and use of laboratory animals.

Phytochemical screening

Identification of the chemical constituents of the aqueous leaf extract of *P. dodecandra* was carried out as per standard procedures by Anees and Mohammed (2004).

Acute toxicity studies (LD_{50}) using Lorke's method (Lorke, 1983)

The test was performed in one phase. Nine rats were randomly selected, placed in three groups each consisting of three rats. For each group, the rats were treated by gavage with varying dose levels of the reconstituted aqueous extract (800, 1280, and 2048 mg/kg). The treated rats were observed for 6 h after administration of the extract for signs of toxicity such as sleepiness, reduced

Table 1. Phytochemical Screening Results of *P. dodecandra*.

Phytochemical	Test method	Deduction
Alkaloids	Picric acid test	Present
Tannins	Ferric chloride test	Present
Phenols	Ferric chloride test	Present
Reducing sugars	Fehling's test	Present
Steroids and triterpenoids	Libermann-Buchard test	Present
Free amino acids	Ninhydrin test	Present

appetite, shivering and general behavior. The rats were further observed for 14 more days for delayed signs of toxicities and deaths.

Determination of abortifacient activity of *P. dodecandra* leaves

The abortifacient activity of the plant extract was tested in pregnant female rats by modification of the method described by Khanna and Chaudhary (1968). Female rats were caged with males of proven fertility in a ratio of 2:1 in the evening and the following day they were checked for evidence of copulation. This day was considered as day 0 of pregnancy.

After ten days, the rats were tested for pregnancy using HCG strips. The pregnant rats were randomly distributed into 5 groups (A to E) of 6 animals each.

On day 15, rats in group A (negative control) were orally administered with distilled water (2 ml), rats in group B (positive control) were administered with misoprostol (100 µg/kg) and those in groups C to E were treated exactly like those in the control groups, but with varying doses of the extract (125, 250 and 500 mg/kg), respectively.

After three days, the animals were anesthetized, dissected and observed for the presence of fetus within the uterus. Absence of fetus in the uterus indicated occurrence of abortion. The number of rats that aborted per group was recorded and the percentage number of rats that aborted per group calculated:

Percentage number of rats that aborted = (number of rats that aborted/total number of rats per group).

Data analysis

Abortifacient activity of the aqueous extract of *P. dodecandra* leaves was determined by the percentage number of rats that aborted per treated group and the overall activity of the extract compared to that of misoprostol (positive control).

Ethical considerations

Due consideration was accorded to guidelines of Faculty of Medicine Ethical Review Committee (Mbarara University of Science and Technology) and international guidelines concerning animal handling (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996) during the research. The experimental animals were put under deep anesthesia using chloroform before dissection and were euthanized using an overdose of chloroform.

The experiment was carried out with respect to the current guidelines for the care of laboratory animals and ethical guidelines (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

RESULTS

Phytochemical screening

Phytochemical screening of aqueous extract indicated the presence of alkaloids, free amino acids, reducing sugars, steroids, triterpenoids, phenols and tannins (Table 1).

Acute toxicity test results

No deaths were observed. However, at the dose level of 2048 mg/kg, signs of toxicity such as reduced appetite, excessive urination, shivering and sleepiness were observed.

Abortifacient activity

In the effect of aqueous extract of *P. dodecandra* on some abortifacient parameters in pregnant Wistar rats as indicated in Table 2, the highest dose of 500 mg/kg had similar effects to that of the standard drug misoprostol at 100 µg/kg.

DISCUSSION

There is much evidence that shows that some herbal drugs can be used as an abortifacient in rats, mice and even humans (Kazerooni et al., 2006; Koneri et al., 2007). A substance that can disrupt pregnancy could be of interest in human fertility control (Goonasekera et al., 1995). Some of these substances can be used as a contraceptive method specifically as a postcoital contraceptive (Bhargava, 1986; Gandhi et al., 1991).

Preliminary phytochemical screening of the leaf extract of *P. dodecandra* indicated the presence of alkaloids, phenols, steroids, reducing sugars, triterpenoids, tannins and free amino acids. These findings are in agreement with phytochemical screening studies carried out on the plant extract by Ogutu (2012) in Kenya. Of these phytochemicals, alkaloids, phenols and steroids are known to possess abortifacient activity (Yakubu and Bukoye, 2009; Khanna and Chaudhury, 1968).

Table 2. Effect of different doses of aqueous extract of *P. dodecandra* leaves on the abortifacient outcome in pregnant wistar rats.

Parameter	Negative control (distilled water)	Positive control (misoprostol 100 µg/kg)	Doses level of extracts used (mg/kg)		
			125	250	500
Number of rats used	6	6	6	6	6
Number of rats that aborted	0 (0%)	6 (100%)	5 (83.3%)	5 (83.3%)	6 (100%)

The aqueous extract of *P. dodecandra* leaves caused toxic signs such as reduced appetite, sleepiness, excessive urination and shivering at the dose of 2048 mg/kg body weight. Therefore, it is toxic, however, the toxicity is dose dependent. According to the toxicity rating chart, this extract is classified as moderately toxic (0.5 to 5 g) per kilogram body weight in humans (Cassarett and Doull, 2008).

On the basis of the aforementioned results three doses (125, 250, and 500 mg/kg body weight) of *P. dodecandra* were selected for the abortifacient studies.

All the experimental extracts when evaluated for their abortifacient activity, were found to exhibit abortifacient activity. The extract at a dose of 500 mg/kg body weight showed 100% abortifacient activity, doses of 125 and 250 mg/kg showed 83.3% abortifacient activity. In some of the animals vaginal bleeding was observed after 4 h of extract administration. This observation reveals that some of the implants were thus aborted. The absence of fetuses in the uteri of the animals where no vaginal bleeding was observed indicated that the implants may have been aborted when the researchers were not around at night or resorbed by the uterus. This indicated post implantation loss of the fetuses proving the pregnancy terminating potential of *P. dodecandra* leaves.

The overall percentage number of rats that aborted after administration of the extract was 91.7% compared to that of misoprostol which was 100% indicating that the extract of *P. dodecandra* leaves has abortifacient activity. In the present study, exposure of pregnant rats to the aqueous extract of *P. dodecandra* leaves at a dose of 500 mg/kg body weight showed 100% abortifacient activity similar to the effect that was observed with the positive control group in which 100 µg/kg body weight misoprostol was administered, indicating that a dose of 500 mg/kg of *P. dodecandra* is as potent in inducing abortion as misoprostol 100 µg/kg.

Preliminary phytochemical studies indicated the presence of alkaloids, steroids, triterpenoids and phenols in *P. dodecandra* leaf extract. Several of these compounds are known to exhibit antifertility activity (Hiremath and Hanumantharo, 1990; Wang and Ruan, 1996).

Alkaloid-like constituent were reported to be responsible for the suppressant effect on the uterine normal contraction and the high anti-implantation activity exhibited by the aqueous extract of *Graptophyllum pictum* (Stella et al., 2009). Alkaloids (Ergot alkaloids) have been known for more than 2000 years to have adverse effect on pregnancy and can be used alone or in combination with oxytocin to induce abortion (Elderfied, 1980).

Phenolics have also been implicated to be promoters of abortion (Saraiya et al., 1998). In agreement with a study carried out by Yakubu and Bukoye (2009) to determine the abortifacient potentials of the aqueous extract of *Bambusa vulgaris* leaves, the extract contained phenolics and alkaloids which were found to be responsible for its abortifacient activity. Sex hormones being steroidal compounds, the plant sterols were suspected to be responsible for the antifertility effects of the leaves of *P. dodecandra* (Khanna and Chaudhury, 1968). This could therefore mean that alkaloids, phenolic compounds and steroids found in the extract of *P. dodecandra* could be responsible for the abortifacient activity that was found.

Conclusion

The aqueous leaf extract of *P. dodecandra* is moderately toxic; when administered orally, possesses abortifacient activity which could be due to the presence of phenolics, alkaloids and steroids in the extract.

Conflict of interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The author acknowledged Ms. Lorna Namuleme for the moral and financial support needed to accomplish this project.

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